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(54) MODEL ANIMAL WITH OVEREXPRESSION OF REGUCALCIN

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a model animal with the overexpression of regucalcin which is inherently expressed in the liver or the like of higher animals, showing bone disease conditions typified by osteoporosis.

SOLUTION: Regucalcin cDNAs are cloned from a rat liver cDNA library and a full-length cDNA encoding the regucalcin protein is isolated. The ORE is excised from the rat regucalcin full-length cDNA and transferred into an expression vector (pCXN2). Then, the gene expression vector is microinjected into the male pronucleus of a fertilized rat ovum. The fertilized ovum is transplanted into the uterine tube of a host rat, and a rat infant is developed. From this newborn child, a homozygous rat is constructed. The transgenic rat exhibits remarkable morphological and biochemical bone disease conditions, and body weight gain is significantly inhibited.

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CLAIMS

[Claim(s)]
[Claim 1] The transgenic nonhuman animal characterized by introducing a REGYUKA rutin gene and carrying out the superfluous manifestation of the REGYUKA rutin.
[Claim 2] A cytomegalovirus-IE enhancer, a chicken beta-actin promotor, a REGYUKA rutin gene, the transgenic nonhuman animal according to claim 1 characterized by introducing the straight chain DNA arranged in order of the rabbit beta globin poly A signal.
[Claim 3] The transgenic nonhuman animal according to claim 1 or 2 characterized by being the gene which carries out the code of the protein with which a REGYUKA rutin gene consists of an amino acid sequence of array number 2 publication of an array table.
[Claim 4] The transgenic nonhuman animal according to claim 3 characterized by being the rat REGYUKA rutin gene which the gene which carries out the code of the protein which consists of an amino acid sequence of array number 2 publication of an array table turns into from the DNA array of array number 1 publication of an array table.
[Claim 5] Claims 1-4 characterized by being a gay object are the transgenic nonhuman animals of a publication either.
[Claim 6] Claims 1-5 characterized by having weight increase control ability are the transgenic nonhuman animals of a publication either.
[Claim 7] Claims 1-6 characterized by being cerebrum functional disorder onset nature are the transgenic nonhuman animals of a publication either.
[Claim 8] Claims 1-7 characterized by being insulin non-dependency diabetes-mellitus onset nature are the transgenic nonhuman animals of a publication either.
[Claim 9] Claims 1-8 characterized by being renal hypertension onset nature are the transgenic nonhuman animals of a publication either.
[Claim 10] Claims 1-9 characterized by being tubular reabsorption failure

onset nature are the transgenic nonhuman animals of a publication either.
[Claim 11] Claims 1-10 characterized by a nonhuman animal being a rat are the transgenic nonhuman animals of a publication either.
[Claim 12] Claims 1-11 are the manufacture approaches of the REGYUKA rutin characterized by using the transgenic nonhuman animal of a publication either.
[Claim 13] either of claims 1-11 -- the screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation characterized by using the transgenic nonhuman animal of a publication or the organization of this transgenic nonhuman animal origin, an organ or a cell, and a specimen material.
[Claim 14] The screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 characterized by medicating a transgenic nonhuman animal with a specimen material, and measuring and evaluating extent of the weight increase in this transgenic nonhuman animal.
[Claim 15] The screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 or 14 whose disease resulting from a REGYUKA overrutin manifestation is characterized by being a cerebrum functional disorder.
[Claim 16] The screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 or 14 whose disease resulting from a REGYUKA overrutin manifestation is characterized by being insulin non-dependency diabetes mellitus.
[Claim 17] The screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 or 14 whose disease resulting from a REGYUKA overrutin manifestation is characterized by being renal hypertension.
[Claim 18] The screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 or 14 whose disease resulting from a REGYUKA overrutin manifestation is characterized by being a tubular reabsorption failure.
[Claim 19] Claims 13-18 are prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation obtained by the screening approach of a publication either.
[Claim 20] Claims 1-11 are the screening approaches of the causative agent of the disease resulting from the REGYUKA rutin manifestation lowering characterized by using the transgenic nonhuman animal of a publication or the organization of this transgenic nonhuman animal origin, an organ or a cell, and a specimen material either.
[Claim 21] The screening approach of the causative agent of the disease

resulting from the REGYUKA rutin manifestation lowering according to claim 20 characterized by medicating a transgenic nonhuman animal with a specimen material, and measuring and evaluating extent of the loss weight in this transgenic nonhuman animal.

[Claim 22] The screening approach of the causative agent of the disease resulting from the REGYUKA rutin manifestation lowering according to claim 20 or 21 whose disease resulting from REGYUKA rutin manifestation lowering is characterized by being arteriosclerosis myocardial infarction.

[Claim 23] The screening approach of the causative agent of the disease resulting from the REGYUKA rutin manifestation lowering according to claim 20 or 21 whose disease resulting from REGYUKA rutin manifestation lowering is characterized by being myocardial infarction.

[Claim 24] Claims 20-23 are the causative agents of the disease resulting from the REGYUKA rutin manifestation lowering obtained by the screening approach of a publication either.

[Claim 25] The bone symptoms model animal which is a nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin, and is characterized by presenting bone symptoms.

[Claim 26] The bone symptoms model animal according to claim 25 characterized by presenting any one or more bone symptoms of the embrittlement of an osseous tissue, bone gestalt change, and bone growth delay.

[Claim 27] The bone symptoms model animal according to claim 25 or 26 characterized by being selected and checked by bony morphological measurement assessment and/or biochemical measurement assessment of a bone component from the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin.

[Claim 28] The bone symptoms model animal according to claim 27 to which bony morphological measurement assessment is characterized by being any one or more measurement assessment of bone density, bone reinforcement, diaphysis cortical bone thickness, and perimeter [cortical bone] die length.

[Claim 29] The bone symptoms model animal according to claim 27 to which biochemical measurement assessment of a bone component is characterized by being any one or more measurement assessment of the amount of calcium, alkaline-phosphatase activity, and the amount of DNA in an osseous tissue.

[Claim 30] Claims 25-29 characterized by the quality of a phenotype of bone symptoms being stable in passage are the bone symptoms model animals of a publication either.

[Claim 31] Claims 25-30 characterized by the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin being a transgenic nonhuman animal into which the REGYUKA rutin gene was introduced are

the bone symptoms model animals of a publication either.
[Claim 32] 26-32 to which the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin is characterized by being a gay object are the bone symptoms model animal of a publication either.
[Claim 33] Claims 25-32 to which the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin is characterized by being a female nonhuman animal are the bone symptoms model animals of a publication either.
[Claim 34] Claims 25-33 to which the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin is characterized by being a rat are the bone symptoms model animals of a publication either.
[Claim 35] either of claims 25-34 -- the screening approach of of the prevention and the remedy of the bone symptoms characterized by medicating the bone symptoms model animal of a publication with a specimen material, and performing morphological measurement assessment of the bone in this bone symptoms model animal, and/or biochemical measurement assessment of a bone component.
[Claim 36] The screening approach of of the prevention and the remedy of bone symptoms according to claim 35 that bony morphological measurement assessment is characterized by being any one or more measurement assessment of bone density, bone reinforcement, diaphysis cortical bone thickness, and perimeter [cortical bone] die length.
[Claim 37] The screening approach of of the prevention and the remedy of bone symptoms according to claim 35 that biochemical measurement assessment of a bone component is characterized by being any one or more measurement assessment of the amount of calcium, alkaline-phosphatase activity, and the amount of DNA in an osseous tissue.
[Claim 38] Claims 35-37 characterized by bone symptoms being osteoporosis are the screening approaches of of the prevention and the remedy of the bone symptoms of a publication either.
[Claim 39] Claims 35-38 are prevention and the remedy of the bone symptoms obtained by the screening approach of a publication either.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the manufacture approach of REGYUKA rutin of using a REGYUKA rutin transgenics transgenic nonhuman animal, the transgenic nonhuman animal which a REGYUKA rutin gene is introduced and has weight increase control ability in detail, and this transgenic nonhuman animal, the screening approach of of the prevention and the remedy of the disease resulting from a REGYUKA overrutin manifestation, the screening approach of the causative agent of the disease resulting from REGYUKA rutin manifestation lowering, etc. Moreover, from the model animal of the bone symptoms represented by osteoporosis, and the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin in more detail, this invention is selected and checked by bony morphological measurement assessment or biochemical measurement assessment of a bone component, and relates to the screening approach of the prevention and the remedy of the bone symptoms represented by the osteoporosis using the bone symptoms model animal which presents bone symptoms, such as embrittlement of an osseous tissue, bone gestalt change, and bone growth delay, and the model animal of these bone symptoms.
[0002]
[Description of the Prior Art] Peptide hormone combines with the acceptor of a cell membrane, and calcium2+ is playing the leading role in the structure which transmits the information to intracellular. Although much protein which combines calcium2+ exists in intracellular, the calmodulin has played the important role as protein which amplifies that operation, calcium2+ is combined with this calmodulin, and activating various kinds of enzymes which participate in accommodation of a cell function is solved (Science, 202, 19-27, 1984). Moreover, it is also known that calcium2+ will act on proteinkinase C or other calcium2+ binding protein (an enzyme is also

included) (Science, 233, 305-312, 1986). It is the calcium2+ binding protein with which REGYUKA rutin was also isolated from the quality of a rat liver cell by this invention persons.

[0003] Molecular weight is calcium2+ binding protein of 33388, and REGYUKA rutin is acidic protein of the isoelectric point pI5.20 which exists in liver notably which the calcium2+ coupling constant shows 4.19x105M-1, has 6-7 high compatibility calcium2+ binding sites, and includes alpha-helix structure 34%. Although REGYUKA rutin is unique protein which does not include the part EF hand structure (field) where a calmodulin and the calcium2+ binding protein of other many see, for example, an alpha-helix content increases a calmodulin and the structure becomes strong by combining calcium2+, as for REGYUKA rutin, an alpha-helix content decreases. Moreover, on the other hand in cell functional accommodation, it is clear for REGYUKA rutin to check the enzyme activation by the calmodulin, and to also check activation of the pro TIN kinase C. Thus, many knowledge -- REGYUKA rutin functions as control protein of signaling -- is accumulated (FEBS Lett, 327, 251-255, 1993).

[0004] A REGYUKA rutin gene exists in an X chromosome (Xq 11.1-12) in a rat, and is located in an X chromosome also in Homo sapiens. Although the REGYUKA rutin gene is found out by higher animals, such as a rat, an ape besides Homo sapiens, a mouse, a dog, a cow, a rabbit, and a fowl, it is not in yeast, and it is thought that the code of the protein which specialized in altitude is carried out. Cloning of the REGYUKA rutin cDNA is carried out, and all the structures are also determined (JP,7-123985,A). The base pairs which carry out the code of all the amino acid are 0.897kbs, and the REGYUKA rutin cDNA of a rat liver translates the amino acid of 299. Moreover, the base sequence of the REGYUKA rutin cDNA of a mouse liver or a Homo sapiens liver is also determined, and it has 94% and about 89% of homology as compared with the REGYUKA rutin cDNA of a rat liver, respectively. The manifestation of REGYUKA rutin mRNA is set to liver, such as Homo sapiens, a rat, a mouse, a cow, and a fowl, and existence of REGYUKA rutin protein is also checked by such liver.

[0005] It is known that REGYUKA rutin is protein which has the description as control protein of intracellular calcium2+ signaling which has various functions nature, and it is the important protein which participates in cell functional accommodation (Life Sciences 66, 1769-1780, 2000, Biochemical and Biophysical Research Communications 276, 1-6, 2000). Moreover, it is shown clearly in animal experiment that the manifestation of the REGYUKA rutin in liver in the living body or the kidney falls at the time of a hepatopathy (Molecular and CellularBiochemisty 131, 173-179, 1994) or renal dysfunction (Molecular and Cellular Biochemisty 151, 55-60, 1995), and the relation of REGYUKA rutin and the symptoms origin is suggested. And while REGYUKA

rutin is going up intentionally, by healthy people's blood serum, most REGYUKA rutin is not detected but it is known for the blood serum of the approach of judging liver disease patient's serum by measuring the concentration in the blood serum of the REGYUKA rutin which exists in liver specifically unlike the existing liver function markers, such as GOT and GPT, i.e., a liver disease patient, that the measurement is also useful as a differentiation means of liver disease patient's serum (JP,10-26623,A).
[0006] On the other hand, it consists of spongin with which the osseous tissue consisted of osteocyte and a substrate, the quality of organic which uses a collagen as a principal component one third, and 2/3 are made of the minerals which are the bone salt of calcium-Lynn, the structure top was divided into the substantia compacta, spongin, and a cortex, for example, the diaphysis of a long bone was surrounded by the substantia compacta, and epiphysis was surrounded by the cortex. Once a bone is formed, after that not the structure that does not change at all but osteogenesis, and osteoclasis balance, the structure and amount are maintained. Therefore, if the balance collapses according to the cause of aging or others, the symptoms of various bone diseases will be shown. Although the absolute magnitude of the ****-jet disease to which myeloma, a lymphoma, etc. are brought by the malignant hypercalcemia which happens owing to, and locality osteoclasis among bone diseases as what occurs by the gastric upset of the osteoclasis to which a calcium salt is eluted in blood from a bone, and a bone is decreasing, osteoporosis without a bony qualitative change etc. is mentioned. These diseases generate a bony pain, becoming the cause of fracture by bony embrittlement is known, and current and these diseases are social-problem-ized with the increment in advanced age population.
[0007] In addition, a hypercalcemia, a hypocalcemia, hyperparathyroidism, Bone diseases, such as rickets, osteomalacia, osteoporosis, and osteopenia, glomerulonephritis, It can use as symptoms model animals, such as kidney diseases, such as glomerulosclerosis, chronic nephritis, and renal failure, a malignant tumor, psoriasis, or those complication. [which can perform break through of these symptoms mechanisms, examination of the therapy approach of a disease, and screening of a remedy] The nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle is known (JP,11-9140,A).
[0008]
[Problem(s) to be Solved by the Invention] The unique manifestation of the REGYUKA rutin protein is carried out at liver, and also it is discovered by the low to the kidney, the heart, and a cerebrum (nerve cell). It is the unique multifunctional protein which will cause physiological abnormalities if it participates in accommodation of an intracellular calcium2+ signaling related cell function and the manifestation falls. The protein isolated from the liver of

a rat until now and an anti-REGYUKA rutin monoclonal antibody are used. The functional analysis is performed. Others [role / of a controlling factor of the above-mentioned calcium signal], A role of accommodation of an intracellular calcium transport enzyme, and an activator of a protease, Accommodation of nucleus functions, such as a role in accommodation of calcium transport of a nucleus, and nucleus DNA degradation, and a role in the nucleus function at the time of liver regeneration, The functional role of the REGYUKA rutin in much biological regulation, such as a role in kidney renal tubule calcium resorption, is clarified by this invention person.

[0009] this invention person in the research process about a break through of the various functional roles of REGYUKA rutin Paying attention to the point of having the specific action in which REGYUKA rutin differs from much of other calcium2+ binding protein, functional accommodation of the various cells in which calcium participates It is thought that it is materialized after balancing with the amount of manifestations of much of other calcium2+ binding protein including the amount of manifestations and calmodulin of REGYUKA rutin in the living body. When the balance of the amount of manifestations of REGYUKA rutin and the amount of manifestations of much of other calcium2+ binding protein collapsed, it decided to investigate change and effect produced to a living body. The technical problem of this invention is to provide a living body with the REGYUKA overrutin manifestation model animal which is a tool for investigating what kind of change and effect arise, when the REGYUKA rutin originally discovered to the liver of a higher animal etc. is made to discover superfluously and balance with much of other calcium2+ binding protein is lost.

[0010] Moreover, although the calcium bone metabolic turnover represented by osteoporosis was started and the ovariectomy rat was conventionally used for aging, prevention of the bone symptoms which occur frequently especially in a woman, and remedy agent development, by the time an ovariectomy animal requires a surgical extraction operation and made bone quantity reduction cause further, it needed breeding for three months or more, and, also as for constraint not only becoming a large sum but technical, and time, there was much research expense. Moreover, although there was an inflammatory (rheumatism) arthritis bone symptoms model animal as other bone symptoms model animals seen in a clinical aspect, in order to make the symptoms of this show by medication, it had a problem physiologically with other side effects. Without requiring the surgical extraction operation of an ovariectomy etc. which can solve the above-mentioned problem again, the technical problem of this invention has an unnecessary breeding period until it makes bone quantity reduction cause further, and is to offer the model animal of the bone symptoms represented by osteoporosis which does not

have a physiological problem with a side effect etc.
[0011]
[Means for Solving the Problem] In order that this invention person may solve the above-mentioned technical problem, cloning of the REGYUKA rutin cDNA is carried out from a rat liver cDNA library. Isolate cDNA which carries out the code of the overall length of REGYUKA rutin protein, and ORF is started from this rat REGYUKA rutin overall length cDNA. Introduce into an expression vector (pCXN2) and the microinjection of this gene expression vector is carried out to rat fertilized egg male pronucleus. Transplant this fertilized egg to the oviduct of an assumed-parents rat, generate a ** rat, and DNA is extracted from the organization of that offspring. The place which checked the rat into which REGYUKA rutin cDNA is built by the PCR method, Five rats (four males, one female) of the gay object which discovers REGYUKA rutin cDNA from the offspring of 29 animals are created, and it finds out that the increment in the weight of this transgenic rat is controlled intentionally, and came to complete this invention.
[0012] moreover, this invention person about the transformation rat which gained REGYUKA overrutin manifestation ability by the above-mentioned REGYUKA rutin transgenics which is not presenting bone symptoms at all on appearance the bone by **** and the pQTC (Peripheral Quantitative Computed Tomography) bone density measuring apparatus for animal study is morphological (bone density --) bone reinforcement, diaphysis cortical bone thickness, and a cortical bone -- a boundary length -- measurement assessment and a bone component are biochemical (the amount of calcium --) The alkaline-phosphatase activity which is the marker enzyme of osteoblast and osteoblast, The place which carried out the amount measurement assessment of DNA which is the number index of cells in an osseous tissue, The embrittlement of the osseous tissue by the osteoclasis (bone-salt dissolution) according [especially on a femur and] to reduction of bone quantity and bone density also morphologically and biochemically, It checks that it is what is stable in passage by finding out as for the characteristic of this REGYUKA overrutin manifestation symptoms model rat, and is equal to presenting remarkable bone symptoms, such as bone gestalt change and coccyx growth delay, at commercial production, and came to complete this invention.
[00013] Namely, the transgenic nonhuman animal (claim 1) characterized by introducing a REGYUKA rutin gene and this invention carrying out the superfluous manifestation of the REGYUKA rutin, The transgenic nonhuman animal (claim 2) according to claim 1 characterized by introducing the straight chain DNA arranged in order of the cytomegalovirus-IE enhancer, the chicken beta-actin promotor, the REGYUKA rutin gene, and the rabbit beta globin poly A signal, The transgenic nonhuman animal (claim 3)

according to claim 1 or 2 characterized by being the gene which carries out the code of the protein with which a REGYUKA rutin gene consists of an amino acid sequence of array number 2 publication of an array table, The gene which carries out the code of the protein which consists of an amino acid sequence of array number 2 publication of an array table The transgenic nonhuman animal (claim 4) according to claim 3 characterized by being the rat REGYUKA rutin gene which consists of a DNA array of array number 1 publication of an array table, Claims 1-4 characterized by being a gay object either The transgenic nonhuman animal (claim 5) of a publication, Claims 1-5 characterized by having weight increase control ability either The transgenic nonhuman animal (claim 6) of a publication, Claims 1-6 characterized by being cerebrum functional disorder onset nature either The transgenic nonhuman animal (claim 7) of a publication, Claims 1-7 characterized by being insulin non-dependency diabetes-mellitus onset nature either The transgenic nonhuman animal (claim 8) of a publication, Claims 1-8 characterized by being renal hypertension onset nature either The transgenic nonhuman animal (claim 9) of a publication, either of claims 1-9 characterized by being tubular reabsorption failure onset nature -- either of claims 1-10 characterized by the transgenic nonhuman animal (claim 10) and nonhuman animal of a publication being a rat -- it is related with the transgenic nonhuman animal (claim 11) of a publication.

[0014] Claims 1-11 this invention either Moreover, the manufacture approach (claim 12) of the REGYUKA rutin characterized by using the transgenic nonhuman animal of a publication, Claims 1-11 either The screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation characterized by using the transgenic nonhuman animal of a publication or the organization of this transgenic nonhuman animal origin, an organ or a cell, and a specimen material A transgenic nonhuman animal is medicated with a specimen material. (Claim 13) The screening approach (claim 14) of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 characterized by measuring and evaluating extent of the weight increase in this transgenic nonhuman animal, The screening approach (claim 15) of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 or 14 whose disease resulting from a REGYUKA overrutin manifestation is characterized by being a cerebrum functional disorder, The screening approach (claim 16) of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 or 14 whose disease resulting from a REGYUKA overrutin manifestation is characterized by being insulin non-dependency diabetes mellitus, The screening approach (claim 17) of of the prevention and the

remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 or 14 whose disease resulting from a REGYUKA overrutin manifestation is characterized by being renal hypertension, The screening approach (claim 18) of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation according to claim 13 or 14 whose disease resulting from a REGYUKA overrutin manifestation is characterized by being a tubular reabsorption failure, It is related with prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation obtained by the screening approach of any of claims 13-18, or a publication (claim 19).

[0015] Furthermore, this invention Claims 1-11 either The screening approach (claim 20) of the causative agent of the disease resulting from the REGYUKA rutin manifestation lowering characterized by using the transgenic nonhuman animal of a publication or the organization of this transgenic nonhuman animal origin, an organ or a cell, and a specimen material, The screening approach (claim 21) of the causative agent of the disease resulting from the REGYUKA rutin manifestation lowering according to claim 20 characterized by medicating a transgenic nonhuman animal with a specimen material, and measuring and evaluating extent of the loss weight in this transgenic nonhuman animal, The screening approach (claim 22) of the causative agent of the disease resulting from the REGYUKA rutin manifestation lowering according to claim 20 or 21 whose disease resulting from REGYUKA rutin manifestation lowering is characterized by being arteriosclerosis myocardial infarction, The screening approach (claim 23) of the causative agent of the disease resulting from the REGYUKA rutin manifestation lowering according to claim 20 or 21 whose disease resulting from REGYUKA rutin manifestation lowering is characterized by being myocardial infarction, It is related with the causative agent (claim 24) of the disease resulting from the REGYUKA rutin manifestation lowering obtained by the screening approach of any of claims 20-23, or a publication.

[0016] And this invention is a nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin again. The bone symptoms model animal (claim 25) characterized by presenting bone symptoms, The bone symptoms model animal (claim 26) according to claim 25 characterized by presenting any one or more bone symptoms of the embrittlement of an osseous tissue, bone gestalt change, and bone growth delay, The bone symptoms model animal (claim 27) according to claim 25 or 26 characterized by being selected and checked by bony morphological measurement assessment and/or biochemical measurement assessment of a bone component from the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin, The bone symptoms model animal (claim 28) according to claim 27 to which bony morphological measurement

assessment is characterized by being any one or more measurement assessment of bone density, bone reinforcement, diaphysis cortical bone thickness, and perimeter [cortical bone] die length, Biochemical measurement assessment of a bone component The amount of calcium, alkaline-phosphatase activity, The bone symptoms model animal (claim 29) according to claim 27 characterized by being any one or more measurement assessment of the amount of DNA in an osseous tissue, Claims 25-29 characterized by the quality of a phenotype of bone symptoms being stable in passage either The bone symptoms model animal (claim 30) of a publication, Claims 25-30 characterized by the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin being a transgenic nonhuman animal into which the REGYUKA rutin gene was introduced either The bone symptoms model animal (claim 31) of a publication, 26-32 to which the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin is characterized by being a gay object either The bone symptoms model animal (claim 32) of a publication, Claims 25-32 to which the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin is characterized by being a female nonhuman animal either The bone symptoms model animal (claim 33) of a publication, The nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin is related with the bone symptoms model animal (claim 34) of any of claims 25-33 characterized by being a rat, or a publication.

[0017] This invention medicates the bone symptoms model animal of any of claims 25-34, or a publication with a specimen material again. The screening approach (claim 35) of of the prevention and the remedy of the bone symptoms characterized by performing morphological measurement assessment of the bone in this bone symptoms model animal, and/or biochemical measurement assessment of a bone component, The screening approach (claim 36) of of the prevention and the remedy of bone symptoms according to claim 35 that bony morphological measurement assessment is characterized by being any one or more measurement assessment of bone density, bone reinforcement, diaphysis cortical bone thickness, and perimeter [cortical bone] die length, Biochemical measurement assessment of a bone component The amount of calcium, alkaline-phosphatase activity, The screening approach (claim 37) of of the prevention and the remedy of the bone symptoms according to claim 35 characterized by being any one or more measurement assessment of the amount of DNA in an osseous tissue, either of claims 35-37 characterized by bone symptoms being osteoporosis -- the screening approach (claim 38) of of the prevention and the remedy of the bone symptoms of a publication, and either of claims 35-38 -- it is related with prevention and the remedy of the bone symptoms obtained by

the screening approach of a publication (claim 39).
[0018]
[Embodiment of the Invention] A REGYUKA rutin gene is introduced, as a transgenic nonhuman animal of this invention, especially if it is the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin, it is not restricted, and it says discovering a lot of REGYUKA rutin intentionally compared with the amount of REGYUKA rutin manifestations of the nonhuman animal of a wild type as carrying out the superfluous manifestation of the REGYUKA rutin here. Moreover, as the above-mentioned nonhuman animal, although a rat, a mouse, a cow, Buta, a fowl, a frog, Homo sapiens, a dog, a rabbit, etc. can be mentioned, a rat is desirable especially. Although an organ is small and there may be a limitation in the analysis of symptoms with the mouse well used as a model animal, for example in rats, such as blood pressure measurement, this becomes possible and becomes very useful as an animal experiment-means for a symptoms break through or gene therapy.
[0019] The transgenic nonhuman animal into which the straight chain DNA arranged in order of the cytomegalovirus-IE enhancer, the chicken beta-actin promotor, the REGYUKA rutin gene, and the rabbit beta globin poly A signal was introduced as a desirable mode of the transgenic nonhuman animal of this invention can be mentioned. For example, if what introduced the REGYUKA rutin overall length cDNA into the expression vector (pCXN2) which has a marker gene, a cytomegalovirus-IE enhancer, a chicken beta-actin promotor, a cDNA insertion site, a rabbit beta globin poly A signal, etc. is used, a transgenic nonhuman animal can be obtained efficiently.
[0020] moreover, as a desirable mode of the transgenic nonhuman animal of this invention The transgenic nonhuman animal which is the gene which carries out the code of the protein with which a REGYUKA rutin gene consists of an amino acid sequence of array number 2 publication of an array table, Although the gene which carries out the code of the protein which consists of an amino acid sequence of array number 2 publication of an array table especially can mention the transgenic nonhuman animal which is the rat REGYUKA rutin gene which consists of a DNA array of array number 1 publication of an array table It is not restricted [rabbit / a mouse besides a rat, a cow, Buta, a fowl, a frog, Homo sapiens, a dog,] especially as the origin of a REGYUKA rutin gene.
[0021] Moreover, the transgenic nonhuman animal which is a gay object can be mentioned as a desirable mode of the transgenic nonhuman animal of this invention. It can be acquired by crossing nonhuman animals, such as a rat which has a chromosome in a hetero, and since the gay object which has this variation chromosome to a gay has more amounts of REGYUKA rutin

manifestations than a hetero object, it is desirable especially as an experiment model animal. Moreover, as a transgenic nonhuman animal of this invention, the increment in weight was intentionally controlled compared with the nonhuman animal of a wild type, namely, can mention suitably the transgenic nonhuman animal which has weight increase control ability. The transgenic nonhuman animal which a REGYUKA rutin gene is introduced and carries out the superfluous manifestation of the REGYUKA rutin having this weight increase control ability is were not able to expect at all, and it has suggested that, as for this new knowledge, REGYUKA rutin may have the usefulness as an obesity inhibitor. Considering this new knowledge, a REGYUKA rutin gene is introduced and the transgenic nonhuman animal of this invention can also be called transgenic nonhuman animal which has the weight increase control ability characterized by carrying out the superfluous manifestation of the REGYUKA rutin.

[0022] Moreover, the transgenic nonhuman animal which discovers at least one or more symptoms and a disease as a desirable mode of the transgenic nonhuman animal of this invention among the symptoms and diseases resulting from the REGYUKA overrutin manifestation of cerebrum functional disorder onset nature, insulin non-dependency diabetes-mellitus onset nature, renal hypertension onset nature, tubular reabsorption failure onset nature, etc. can be mentioned. The REGYUKA rutin which carried out the superfluous manifestation of the activation of calcium-calmodulin dependency protein kinase needed on the storage maintenance mechanism of a cerebrum controls a cerebrum functional disorder, it is thought that symptoms develop by controlling the neural transmission in a nerve cell, and the transgenic nonhuman animal of this invention is useful as an experiment model animal of the failure (Alzheimer's disease, such as Alzheimer) of cerebration, such as storage. In liver or the kidney, it is discovered and REGYUKA rutin is controlling intracellularsignal transduction of hormone. Moreover, by the superfluous manifestation of REGYUKA rutin The failure of the operation manifestation of hormone which adjusts the function of liver and the kidney is carried out, and it sets to liver. Since work of an insulin is controlled, induce insulin non-dependency diabetes mellitus, and it sets into the kidney. It is thought that the renal hypertension related to a renin-angiotensin series and the tubular reabsorption failure further relevant to the electrolyte metabolism are induced. The transgenic nonhuman animal of this invention It is useful as experiment model animals, such as insulin non-dependency diabetes mellitus, renal hypertension, and a tubular reabsorption failure.

[0023] As the establishment approach of model animals, such as a model rat which has the weight increase control ability of this invention, the approach using the production approach (7384 for example, Proc.Natl.Acad.Sci.USA

77:7380- 1980) of a well-known transgenic animal can be mentioned. for example, as an approach of inventing a REGYUKA rutin (RC) transgenic rat Cloning of the cDNA of REGYUKA rutin is carried out from a rat liver cDNA library. An open reading frame (ORF) is started after isolating cDNA which carries out the code of the overall length of REGYUKA rutin protein. Introduce into an expression vector and the microinjection of the straight chain DNA fragment containing the introductory gene which carried out the linear rise of this gene expression vector is carried out to rat fertilized egg male pronucleus. This fertilized egg or 2 cell term germ is transplanted to the oviduct of an assumed-parents rat, a ** rat is generated, and the approach of checking REGYUKA rutin cDNA being incorporated by the PCR method etc. using DNA extracted from the organization of that offspring etc. can be mentioned.

[0024] As the manufacture approach of the REGYUKA rutin of this invention, especially if it is the transgenic nonhuman animal of this invention, and an approach using the transgenic nonhuman animal of a gay object preferably, it is not restricted, and it applies to the approach of ejection and the reference (Chem.Pharm.Bull.26, 1915-1918, 1978) publication from the homogenate of liver correspondingly from the REGYUKA rutin transgenic rat of a gay object, for example, REGYUKA rutin can be isolated and refined. Moreover, a transgenic nonhuman animal can also be medicated with calcium, a cultivator tonin, an insulin, estrogen, etc. for the purpose of increase of income of REGYUKA rutin.

[0025] It is not restricted especially if it is an approach using the transgenic nonhuman animal of this invention or the organization of this transgenic nonhuman animal origin, an organ or a cell, and a specimen material as the screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation of this invention, and as a disease resulting from the above-mentioned REGYUKA overrutin manifestation, a cerebrum functional disorder, insulin non-dependency diabetes mellitus, renal hypertension, a tubular reabsorption failure, etc. can be illustrated. As an approach using the above-mentioned transgenic nonhuman animal and a specimen material Extent of weight increase [in / a transgenic nonhuman animal is directly medicated with a specimen material, and / this transgenic nonhuman animal], The approach of measuring and evaluating extent of the disease resulting from a REGYUKA overrutin manifestation, The approach of measuring and evaluating extent of the amount of manifestation control of the REGYUKA rutin in the organization, organ, or cell obtained from the transgenic nonhuman animal after specimen material administration, How the immunity staining technique and electron microscope by the monoclonal antibody estimate the gestalt change in an organization or an organ can be mentioned. Moreover, the organization, the

organ, or the cell of the transgenic nonhuman animal origin can cultivate under existence of a specimen material, and how the immunity staining technique and the electron microscope by the monoclonal antibody estimate the gestalt change in the approach, the organization, and the organ which measure and evaluate extent of the amount of manifestation control of the REGYUKA rutin of this organization, an organ, or a cell can mention as an approach using the organization, the organ or the cell, and the specimen material of the transgenic nonhuman animal origin.

[0026] Hepatocyte, a nerve cell, etc. which constitute liver, a kidney renal tubule, the heart, a cerebrum, etc. as the above-mentioned organization or an organ, and constitute these organizations and an organ as a cell can be mentioned concretely. Moreover, it is desirable from the ability to carry out exact comparative experiments on individual level to compare and estimate it as the case in a wild type nonhuman animal, especially the wild type nonhuman animal of a brood on the occasion of these screening. Thus, according to the screening approach of above-mentioned this invention, prevention and remedies, such as the disease resulting from a REGYUKA overrutin manifestation, for example, a cerebrum functional disorder, insulin non-dependency diabetes mellitus, renal hypertension, and a tubular reabsorption failure, can be screened, and prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation obtained by the starting screening approach are also contained under the category of this invention.

[0027] It is not restricted especially if it is an approach using the transgenic nonhuman animal of this invention or the organization of the transgenic nonhuman animal origin, an organ or a cell, and a specimen material as the screening approach of the causative agent of the disease resulting from REGYUKA rutin manifestation lowering of this invention, and arteriosclerosis, myocardial infarction, etc. can be illustrated as a disease resulting from REGYUKA rutin manifestation lowering. As an approach using the above-mentioned transgenic nonhuman animal and a specimen material Extent of a loss weight [in / a transgenic nonhuman animal is directly medicated with a specimen material, and / this transgenic nonhuman animal], The approach of measuring and evaluating extent of the disease resulting from REGYUKA rutin manifestation lowering, The approach of measuring and evaluating extent of the manifestation augend of the REGYUKA rutin in the organization, organ, or cell obtained from the transgenic nonhuman animal after specimen material administration, How the immunity staining technique and electron microscope by the monoclonal antibody estimate the gestalt change in an organization or an organ can be mentioned. Moreover, as an approach using the organization, the organ or the cell, and the specimen material of the transgenic nonhuman animal origin, the organization, the

organ, or the cell of the transgenic nonhuman animal origin can be cultivated under existence of a specimen material, and how the immunity staining technique and the electron microscope by the monoclonal antibody estimate the gestalt change in the approach, the organization, and the organ which measure and evaluate extent of the manifestation augend of the REGYUKA rutin of this organization, an organ, or a cell can mention.

[0028] Hepatocyte, a nerve cell, etc. which constitute liver, a kidney renal tubule, the heart, a cerebrum, etc. as the above-mentioned organization or an organ, and constitute these organizations and an organ as a cell can be mentioned concretely. Moreover, it is desirable from the ability to carry out exact comparative experiments on individual level to compare and estimate it as the case in a wild type nonhuman animal, especially the wild type nonhuman animal of a brood on the occasion of these screening. Thus, the disease which originates in REGYUKA rutin manifestation lowering according to the screening approach of above-mentioned this invention, For example, the causative agent of the disease resulting from the REGYUKA rutin manifestation lowering which can screen causative agents, such as arteriosclerosis and myocardial infarction, and is obtained by this screening approach By screening the matter which checks the operations, such as matter which it is useful when clarifying further an operation and role of REGYUKA rutin in the living body, and is combined with these causative agents Considering the ability to develop prevention and the remedy of the disease resulting from REGYUKA rutin manifestation lowering, it is useful and the starting causative agent is also contained under the category of this invention.

[0029] Next, it is not restricted, especially if it is the nonhuman animal which carries out the superfluous manifestation of the REGYUKA rutin as a bone symptoms model animal of this invention and is the model animal which presents bone symptoms, and the transgenic nonhuman animal of above-mentioned this invention into which the REGYUKA rutin gene was introduced can be suitably illustrated as this bone symptoms model animal. Therefore, although the screening approach of of the prevention and the remedy of the bone symptoms model animal of this invention or bone symptoms is explained below, in a detail, the publication about the publication about the transgenic nonhuman animal of above-mentioned this invention, the screening approach of of the prevention and the remedy of the disease resulting from the REGYUKA overrutin manifestation of this invention, etc. can be referred to more. In addition, in this invention, bone symptoms mean bones, such as reduction in bone quantity, embrittlement of an osseous tissue, bone gestalt change, and bone growth delay, and the condition the growth is not normal by the calcium bone metabolic error represented by osteoporosis.

[0030] As a bone symptoms model animal of above-mentioned this invention, REGYUKA rutin from the nonhuman animal which carries out a superfluous manifestation Any one or more measurement assessment of bony morphological measurement assessment, for example, bone density, bone reinforcement, diaphysis cortical bone thickness, and perimeter [cortical bone] die length, and/ Or the biochemical measurement assessment of calcium of a bone component, for example, the amount, alkaline-phosphatase activity, Were selected and checked by any one or more measurement assessment of the amount of DNA in an osseous tissue. The bone symptoms model animal which presents any one or more bone symptoms of the embrittlement of an osseous tissue, bone gestalt change, and bone growth delay is desirable. For morphological measurement assessment of the above-mentioned bone The pQTC (Peripheral Quantitative Computed Tomography) bone density measuring apparatus for animal study (Bone Vol.29, No.2, and August 2001; 101-104) can be used especially advantageously. Moreover, biochemical measurement assessment of a bone component can be carried out with the conventional method in this field that is indicated by the example mentioned later. In addition, the bone symptoms model animal checked [which were checked and was above-selected] means the animal with which bony morphological measurement assessment and biochemical measurement assessment of a bone component were presented, the animal of a brood, or its descendant from the ability of the sample offering animal which needs the bone itself, such as a femur, for bony morphological measurement assessment or biochemical measurement assessment of a bone component not to be used as a bone symptoms model animal as it is.

[0031] The bone symptoms model animal of this invention is started as mentioned above, for example from the rat REGYUKA rutin expression vector which this invention person produced. The DNA fragment by which the linear rise was carried out is poured into the fertilized egg follicular ovarian cells adjusted separately by the microinjection method. Generating transplants the germ in which the abnormalities in progress are not accepted in the oviduct of assumed parents after cultivating an ootid. By performing the result of morphological measurement assessment of the bone by the pQTC bone density measuring apparatus for animal study, and biochemical measurement assessment of a bone component especially about the produced offspring What it could select and check, and the quality of a phenotype of bone symptoms was stabilized in passage also in these, and was suitable for commercial production is desirable. Moreover, as a bone symptoms model animal of this invention, the REGYUKA rutin transgenic bone symptoms model animal which is a gay object can be illustrated preferably. It can be acquired by crossing nonhuman animals, such as a rat

which has a chromosome in a hetero, and since the gay object which has this variation chromosome to a gay has more amounts of REGYUKA rutin manifestations than a hetero object, it is desirable from the quality of a phenotype of bone symptoms, such as bone change, appearing more strongly. Furthermore, as a bone symptoms model animal of this invention, a REGYUKA rutin gene is on an X chromosome, and since the quality of a phenotype of bone symptoms, such as bone change, appears more notably in a female rather than a male, the bone symptoms model animal of females, such as a female rat, can be illustrated preferably.

[0032] As the screening approach of of the prevention and the remedy of the bone symptoms of this invention A synthetic compound well-known as not the thing that will be restricted especially if it is the screening approach characterized by medicating the bone symptoms model animal of above-mentioned this invention with a specimen material, and performing morphological measurement assessment of the bone in this bone symptoms model animal, and/or biochemical measurement assessment of a bone component but a specimen material, The organization extract of mammalian, cell culture supernatant liquid, etc. the extract component of various vegetation, etc. are used else [, such as a peptide and protein]. For example, prevention and the remedy of bone symptoms, such as osteoporosis, can be screened by medicating the bone symptoms model animal of this invention with a sample compound taking-orally-wise or parenterally, and carrying out biochemical measurement assessment of bone components, such as morphological measurement assessment of bones, such as bone density, bone reinforcement, diaphysis cortical bone thickness, and perimeter [cortical bone] die length, and for example, the amount of calcium, alkaline-phosphatase activity, the amount of DNA in an osseous tissue, in this bone symptoms model animal. Moreover, it is desirable from the ability to carry out exact comparative experiments on individual level to compare and estimate it as the case in a wild type nonhuman animal, especially the wild type nonhuman animal of a brood on the occasion of these screening.

[0033] Moreover, when it is not restricted especially if it is prevention and the remedy of the bone symptoms obtained by the screening approach of above-mentioned this invention as prevention and a remedy of the bone symptoms of this invention, and using these prevention and a remedy as drugs, various combination components for dispensing, such as the usual support permitted pharmacologically, a binder, a stabilizing agent, an excipient, a diluent, a buffer for pH, disintegrator, a solubilizing agent, a solubilizing agent, and an isotonicity agent, can be added. Suitable above-mentioned prevention and remedy using these prevention and a remedy of a dose which balanced a patient's sex, weight, and symptom in the prevention / therapy approach of bone symptoms, such as osteoporosis, can

be prescribed for the patient taking-orally-wise or parenterally. That is, parenteral administration of what could prescribe for the patient in taking orally by pharmaceutical forms, such as the administration gestalt usually used, for example, powder, granulation, a capsule, syrups, and suspension, or was made into pharmaceutical forms, such as a solution, an emulsion, and suspension, can be carried out with the mold of injection, and also a medicine can also be prescribed for the patient in a nostril with the mold of spray.
[0034]
[Example] Hereafter, although an example explains this invention more concretely, the technical range of this invention is not limited to these instantiation.
Example 1 [rat RCcDNA preparation]
(Preparation of RNA) Liver was extracted from the Wistar system male rat (3 weeks old), and it homogenized with guanidine-isothiocyanate liquid (4M guanidinium thiocyanate, 25mM sodium citrate (pH7.0), 0.5% sarcosyl, 0.1M 2-mercaptoethanol, 2M sodium acetate). This was extracted by phenol-chloroform-isoamyl alcohol mixture and it carried out centrifugal by 4 degrees C and 10,000xg for 20 minutes. Isopropanol was added to the water layer, it was left at -20 degrees C, and RNA was settled. The collected precipitation was dissolved in 0.5% sodium dodecyl sulfate which carried out diethylpyrocarbonate processing. Through and (Pori A) +RNA were refined for this to the oligo (dT) cellulose column.
[0035] (Production of a cDNA library) The Moloney-Murine Leukemia virus reverse transcriptase of 50unit(s) and an oligo (dT) 18 primer linker were added to refined (Pori A) +RNA (5microg), and single-strand cDNA was compounded. Escherichia coli ribonuclease H and DNA polymerase I was added to single-strand cDNA furthermore compounded, and the 2 chain cDNA was compounded. The EcoRI adapter was added to this and it connected with the phage expression vector (lambdaZAPII) digested by XhoI and EcoRI. Furthermore, packaging was carried out to phage using the packaging extract, and the phage of a cDNA library was produced.
[0036] (Selection of a RCcDNA clone) About 1x106 phage of the cDNA library of a rat liver was mixed with Escherichia coli, and inoculation was carried out to 20 agar plates. After half[3 hours and]-incubating at 42 degrees C, the nitrocellulose membrane processed by 10mM isopropyl thio beta-D-galactoside was put on the plate, and it half[3 hours and]-incubated at 37 degrees C. The nitrocellulose membrane incubated at anti-RC rabbit blood serum (x200) and the room temperature for 2 hours, after blocking. After washing the film, it added the alkaline-phosphatase joint anti-rabbit IgG antibody, and incubated. Coloring liquid (a 0.35mM nitroblue tetrazolium, 0.4mM5-BUROMO-4-chloro-3-indolyl phosphate) was made to dip and color this, and the RCcDNA positivity plaque was identified.

[0037] (Subcloning to a plasmid vector) The cDNA fragment of RC with which cloning of the phage vector lambdaZAPII was carried out to lambdaZAPII including the base sequence of pBluescript which is a plasmid vector during that array is inserted in this pBluescript. Moreover, the origin of replication and the ending point of a helper phage exist in the ends of pBluescript. Then, you isolate phage, made it infected with Escherichia coli SURE with R408 helper phage, pBluescript containing the cDNA fragment of RC was made to compound within Escherichia coli, and it was made to emit to the Escherichia coli outside of the body in the form of a helper phage from the identified plaque. This phage liquid was further infected with Escherichia coli SURE, and it was made to reproduce within a bacillus as a plasmid which has the cDNA fragment of RC. Inoculation of this Escherichia coli was carried out to LB plate of 50microg [/ml] ampicillin content, and the ampicillin resistance colony was chosen.
[0038] (Decision of the base sequence of a cDNA insertion) All the base sequences of a cDNA insertion were determined using the Sequenase system (product made from US Biochemical). That is, plasmid DNA was cut by EcoRI, and the fragment added and carried out annealing of the primer, after carrying out alkali denaturation processing. After adding 35S dCTP, 0.1M DTT, and the enzyme liquid for Sequense to this, it divided into four equally, and ddATP, ddGTP, ddTTP, and ddCTP were added to each, and 37 degrees C incubated for 5 minutes. It dissociated by acrylamide gel electrophoresis, and these performed autoradiography, and read the base sequence. All the base sequences of REGYUKA rutin cDNA are shown in the array number 1. Moreover, the acquired amino acid sequence is also shown in the array number 2. The molecular weight of the REGYUKA rutin calculated from now on was 33,388. This value was in agreement with the molecular weight which computed refined REGYUKA rutin with the SDS polyacrylamide electrophoresis method.
[0039] Example 2 [an invention of a transgenic rat]
(Construction of an introductory gene) The DNA fragment containing all ORF(s) was cut down using PstI from the plasmid containing the rat REGYUKA rutin overall length cDNA obtained in the example 1, RC-900 (glycerol stock;RC-F), and Vector pBluescript SK (-) (drawing 1 A). This started PstI fragmentation was included in the PstI site of pBluescript II KS (+) (drawing 1 B). Next, it started by EcoRI, the obtained EcoRI fragmentation (drawing 2 A) was introduced into the EcoRI site of an expression vector pCXN2 (Clontech) (Gene [108], 193-199, 1991) (drawing 2 B), and the rat REGYUKA rutin expression vectors RC/pCXN2 were prepared. This RC/pCXN2 was cut by SalI, SfiI, and MluI, and the fragmentation of 3.6kbp(s) by which the linear rise was carried out was obtained (drawing 3).

[0040] (Production of a transgenic rat) The microinjection of the DNA fragment solution of 3.6kbp(s) by which the linear rise was carried out [above-mentioned] to the pronucleus term fertilized egg of a rat was carried out in the following way. The 4-weeks old Sprague-balanced-full-trailer (SD, Sprague-Dawley) system female rat was bred for light-and-darkness cycle 12 hours (4:00 - ****** 16:00) at the temperature of about 23 degrees C, and about 55% of humidity, female sexual cycle was observed by the vagina smear, and the hormone processing date was chosen. After having injected intraperitoneally the pregnant mare serum gonadotropin (Japanese all "PMS ZENYAKU" by the medicine company) of 150 IU/kg to the female rat, performing superovulation processing and injecting intraperitoneally the Homo sapiens choriogonadotropin (the "PU ** low gene" by Sankyo Yell Yakuhin) of 150 IU/kg the 48 hours after, it was made to cross by living together with a male, and the pronucleus term fertilized egg was extracted by oviduct perfusion 32 hours after Homo sapiens choriogonadotropin administration.

[0041] Thus, micro impregnation of said DNA fragment solution (5 ng/mu l concentration) of 3.6kbp(s) was carried out at the male pronucleus of the fertilized egg of the prepared Wistar rats. The egg with which the DNA fragment was poured in was cultivated one evening using the m-KRB (m-Krebs ringer buffer solution) culture medium within the CO2 incubator. Generating progressed to two cell on the next day, about 20-30 per animal were transplanted for 2 cell term germ in which abnormalities are not accepted in the oviduct of assumed parents (a vasoligature male and pseudopregnancy female rat made to cross) of nine animals, and the offspring of 29 animals was obtained. To 4 weeks old, from the tail of the offspring of 27 animals which survived, DNA was extracted and extracted DNA was authorized by the PCR method using primer huRC-1;GGAGGCTATGTTGCCACCATTGGA (array number 3) and primer huRC-2;CCCTCCAAAGCAGCATGAAGTTG (array number 4) (drawing 4). Consequently, existence of an introductory gene was checked to a total of five rats (four males, one female). Five animals told the introductory gene to the next generation among those.

[0042] Example 3 [weight increase control ability]

The transgenic rat (gay object) was obtained by crossing systems with most amounts of REGYUKA rutin manifestations in a tail organization among the systems of the transgenic rat (hetero object) obtained in the example 2. Moreover, that it is a gay object checked inclusion of the introductory gene to the genomic DNA extracted from the rat tail organization in the PCR method, and it checked it by detecting the amount of cDNA(s) twice [more than] the amount of inclusion of a hetero object. It investigated about weight increase control ability using the transgenic rat of this gay object. a

3-4-weeks old wild type Sprague-Dawley rat and a transgenic rat (gay object) -- the average of the weight of every eight animals is shown in a table 1, respectively. It expressed with Student's t test, P< 0.01, and average-value ** standard variation, the significant difference was accepted, and it was able to check that weight increase was controlled by the superfluous manifestation of a REGYUKA rutin gene.

[0043]

[A table 1]

ラットの体重（g）

	体重（g）
Wild	88.5±3.8
Transgenic	69.5±2.4*

[0044] Example 4 [a bone symptoms model animal]

(SD system gay type bone symptoms model rat) The transgenic rat (gay object) was obtained by crossing systems with most amounts of REGYUKA rutin manifestations in a tail organization among the systems of the transgenic rat (hetero object) obtained in the example 2. Moreover, that it is a gay object checked inclusion of the introductory gene to the genomic DNA extracted from the rat tail organization in the PCR method, and it checked it by detecting the amount of cDNA(s) twice [more than] the amount of inclusion of a hetero object. Out of the transgenic rat of the above-mentioned gay object which is not presenting appearance top bone symptoms It is stabilized in **** and the rat of the sex which survives is used as an SD system bone symptoms model rat (gay object) of an experimental plot. bony morphological measurement assessment (bone density, bone reinforcement, diaphysis cortical bone thickness, and a cortical bone -- a boundary length) and biochemical measurement assessment (the amount of calcium, the alkaline-phosphatase activity which is the marker enzyme of osteoblast and osteoblast, the amount of DNA which is the number index of cells in an osseous tissue) of a bone component were performed. Moreover, SD system wild type normal rat of a sex was used as a control plot. In addition, in each measurement assessment, the rat used each five groups, and the experimental plot and the control plot showed each measured value according to the average ** standard error, performed statistical significance detection using Student's t-test, and made below P< 0.01 (1%) those with a significant difference.

[0045] (Bony morphological measurement assessment) SD system bone symptoms model rat (experimental plot) of a 5-6-weeks old sex and SD system wild type normal rat (control plot) were dissected under anesthesia, the muscular system was removed after extracting a femur organization, and

what dipped thoroughly and was saved in 70% ethanol solution was used as the sample until it presented predetermined measurement. Five scans were performed [in / for this sample / the metaphysis section] by slice width of face of every 0.5mm from the 2.0mm part from the distal epiphysis (epiphyseal plate) using the pQTC bone density measuring apparatus for animal study (XCT Reserch SA+:Stratec Medizintecnik GmbH Pforzhein Germany). Moreover, the part of bone length's abbreviation 1/2 was made into diaphysis, and the one-place scan was performed. The result of a scan is shown in drawing 5 (the metaphysis section and the lower-berth left diaphysis) and drawing 6 (the metaphysis section and the lower-berth left diaphysis). [an upper case and the middle] [Experimental plot;] [an upper case and the middle] [Control plot;] The bone density of each group diaphysis and the metaphysis section, bone reinforcement, the cortical bone thickness of a diaphysis organization, and the cortical bone adventitia boundary length of a diaphysis organization were automatically computed and displayed after the scan. A result is shown in a table 2 - a table 5, respectively. In addition, the measurement parameter and solution parameter in the above-mentioned pQTC measurement are shown in a table 6.

[0046] It was remarkable in [by the bone symptoms model rat, bone density decreases / male and female / a normal rat /, especially] the female as a result of pQTC measurement (table 2). In bone reinforcement, although bone reinforcement was decreasing by the male bone symptoms model rat as compared with the male normal rat, in the female, it became clear that bone reinforcement decreases to about 40 - 45% of a normal rat by the bone symptoms model rat in diaphysis and the metaphysis section (table 3). As for the cortex of a diaphysis (cortical bone) organization, cortical bone thickness was decreasing intentionally by the bone symptoms model rat [male and female / a normal rat] (table 4). Although the significant difference was not accepted for the cortex adventitia boundary length of a diaphysis (cortical bone) organization by 2 between groups in the male, in the female, the cortex adventitia boundary length decreased intentionally by the bone symptoms model rat as compared with the normal rat (table 5).

[0047]

[A table 2]

骨組織の骨密度 (mg/cm³)

		骨幹部 (mg/cm³)	骨幹端部(mg/cm³)
Male	Wild	494.3±12.94	345.8±12.25
	Transgenic	425.0±31.28*	304.4±19.69*
Female	Wild	465.8±15.05	388.0±18.77
	Transgenic	216.0±5.38*	274.6±7.82*

[0048]

[A table 3]
骨組織の骨強度（mm^3）

		骨幹部（mm^3）	骨幹端部（mm^3）
Male	Wild	2.794±0.127	3.426±0.077
	Transgenic	2.368±0.308	3.012±0.394
Female	Wild	2.446±0.063	3.194±0.102
	Transgenic	1.163±0.029*	1.298±0.108*

[0049]
[A table 4]
骨幹部（皮質骨）組織の皮質骨厚（mm）

		骨幹部（mm）
Male	Wild	0.309±0.012
	Transgenic	0.112±0.016*
Female	Wild	0.337±0.012
	Transgenic	0.257±0.040*

[0050]
[A table 5]
骨幹部（皮質骨）組織の皮質骨外膜周囲長（mm）

		骨幹部（mm）
Male	Wild	9.365±0.183
	Transgenic	9.540±0.175
Female	Wild	9.004±0.096
	Transgenic	8.761±0.234*

[0051]
[A table 6]
測定パラメーター及び測定部位

スライス厚	500μm	レファレンスの位置	ＳＶ画像から大腿骨遠端部を指定
ボクセルサイズ	80μm	測定部位：骨幹端部（海綿骨）	遠位骨端より 2.0mmから0.5mmごとに計５スライス
	80μm	測定部位：骨幹部（皮質部）	骨長の約１／２の部位
測定時間	１検体当たり約７分　（ＳＶスキャン含む）		

解析パラメーター

CALCBD				CORTBD (SSI)	
Contour mode:2		Peel mode:2		Cortical mode:1	
Threshold:	- - -	Trab. Area:	- - -	Threshold:	690/(&464)mg/cm³
		Threshold:	395mg/cm³	Inner Threshold:	

[0052] (Biochemical measurement assessment of a bone component) SD system gay type bone symptoms model rat (experimental plot) of a 5-6-weeks old sex and SD system wild type normal rat (control plot) were dissected under anesthesia, the muscular system was removed after extracting a femur organization, and what dipped thoroughly and was saved in 70% ethanol solution was used as the sample until it presented predetermined measurement. From this sample, it divided into diaphysis (cortical bone) and the metaphysis section (cancellous bone), and the amount of calcium, the alkaline-phosphatase activity which is the marker enzyme of osteoblast and osteoblast, and the amount of DNA which is the number index of cells in an osseous tissue were measured.
[0053] Diaphysis (cortical bone) and the metaphysis section (cancellous bone) were ashed at 640 degrees C for 24 hours, respectively, weight was measured, it dissolved in 6-N hydrochloric acid after that, and measurement of the amount of calcium in an osseous tissue (mg/g bone dry weight) measured the amount of bone calcium by whenever [atomic absorption]. The result of having expressed the amount of calcium in an osseous tissue with mg/g bone dry weight is shown in a table 7. Although the amount of bone calcium was decreasing intentionally by the bone symptoms model rat [male and female / a normal rat] as shown also in a table 7, reduction of the amount of bone calcium was remarkable in especially the female.
[0054]
[A table 7]
骨組織中カルシウム量（ｍｇ／ｇ骨乾燥重量）

		骨幹部	骨幹端部
Male	Wild	217.6±4.47	169.1±3.99
	Transgenic	192.0±7.89*	142.5±2.46*
Female	Wild	219.4±3.51	185.4±8.55
	Transgenic	174.4±4.69*	137.3±8.54*

[0055] Measurement of the alkaline phosphatase activity in an osseous tissue dipped diaphysis (cortical bone) and the metaphysis section (cancellous bone) in 3ml (pH7.4) of 6.5mM PARUBI tar buffer solutions ice-cooled, respectively, cut them into the wafer, was made into homogeneity with the Potter-Elvehjem homogenizer which the Teflon (trademark) pestle attached, and it was destroyed, having applied it for 60 seconds with the ultrasonic device. The supernatant liquid obtained in 600rpm by carrying out at-long-intervals alignment separation for 5 minutes was used for measurement of enzyme activity. Alkaline phosphatase activity was measured according to the approach (Bergmeyer HU (ed) Methodsof enzymatic analysis, Vol.1-2, Academic Press, New York, PP 856-860, 1965) of Walter and Schutt. Moreover, proteinic concentration was measured

according to Lowry's and others approach (J.Biol.Chem., 193, 265-273, 1951). The result of having expressed the alkaline phosphatase activity in an osseous tissue as mumol/min/mg protein of p-nitrophenol which separated is shown in a table 8. As compared with the normal rat, by the bone symptoms model rat, in diaphysis (cortical bone), alkaline phosphatase activity was rising intentionally by the male, and alkaline phosphatase activity was rising intentionally for the female in the metaphysis section (cancellous bone) from a table 8.

[0056]

[A table 8]

骨組織中アルカリ性ホスファターゼ活性（μｍｏｌ／分／ｍｇ蛋白質）

		骨幹部	骨幹端部
Male	Wild	1.467±0.072	1.246±0.038
	Transgenic	1.104±0.093*	1.204±0.038
Female	Wild	1.192±0.076	1.355±0.029
	Transgenic	1.067±0.095	1.107±0.011*

[0057] After it dipped diaphysis (cortical bone) and the metaphysis section (cancellous bone) in 3ml (pH7.4) of 6.5mM PARUBI tar buffer solutions ice-cooled, respectively and measurement of the amount of DNA in an osseous tissue cut them into the wafer, it was shaken for 24 hours by 4.0ml of ice-cooled 0.1-N sodium-hydroxide solutions. At-long-intervals alignment separation was carried out by 10,000rpm after the alkali extract for 5 minutes, and the obtained supernatant liquid was used for measurement of the amount of DNA. The amount of DNA was measured according to the approach (77 J. Biol.Chem., 214, 39- 1955) of Ceriotti. The result of having expressed the amount of DNA in an osseous tissue as mg/g osseous tissue wet weight is shown in a table 9. As compared with the normal rat, by the bone symptoms model rat, in diaphysis (cortical bone), the amount of DNA was decreasing intentionally for the female, and, in the sex, the amount of DNA was decreasing intentionally in the metaphysis section (cancellous bone) from a table 9.

[0058]

[A table 9]

骨組織中ＤＮＡ量（ｍｇ／ｇ骨組織湿重量）

		骨幹部	骨幹端部
Male	Wild	2.55±0.13	4.64±0.29
	Transgenic	2.99±0.24	3.19±0.22*
Female	Wild	2.40±0.31	4.39±0.40
	Transgenic	1.26±20.18*	2.37±0.38*

[0059] as mentioned above, a clear bone change of a femur organization finds out in the bone symptoms model animal of this invention -- having --

this bone change -- the diaphysis (cortical bone) of a femur, and Ryobe, the metaphysis section (cancellous bone), -- setting -- a morphological list -- being biochemical (bone component) -- it accepted, the osseous tissue caused osteoclasis (bone-salt dissolution), and it became clear to be based on the failure also of the osteogenesis being carried out. Especially, the bone change was more remarkable in the female (female) than the male (male). moreover, the bone symptoms model animal of this invention -- setting -- the manifestation of bone symptoms -- it is checked that the characteristic is also stable in ****.

[0060]

[Effect of the Invention] The REGYUKA rutin transgenic nonhuman animal, especially REGYUKA rutin transgenic rat of this invention are useful as experiment model animals for symptoms assessment, such as an adult disease in which calcium2+ signaling, such as a hepatopathy, renal dysfunction, diabetes mellitus, myocardial infarction, hypertension, and Alzheimer, participates, a lifestyle-related disease, and geriatric diseases. moreover, an organ since REGYUKA rutin is adjusting the cell function relevant to intracellular calcium2+ signaling and the REGYUKA rutin transgenic nonhuman animal of this invention carries out the superfluous manifestation of this REGYUKA rutin -- it can become a means useful as a model animal for the gene therapy medicine development for restoration and an improvement of specific symptoms (hepatic carcinoma, myocardial infarction, cerebrum Alzheimer's disease). Moreover, the bone symptoms model animal of this invention can be used in favor of the preclinical test aiming at a break through of a bone symptoms device, or development of a new drug etc. as a symptoms model animal for bone disease therapies, such as osteoporosis.

[0061]

[Layout Table]

SEQUENCE-LISTING <110> JAPAN SCIENCE AND-TECHNOLOGY-CORPORATION<120> Regucalcin-gene-transferred-non-human-animals<130> 13-217<140><141><160> 4 <170> PatentIn Ver. 2.1<210> 1<211> 900<212> DNA<213> Rattus norvegicus<220> <221>CDS <222> (1) .. (900) <400> 1 atg tct tcc atc aag att gaa tgt gtt tta agg gag aac tac agg tgt 48 Met Ser Ser Ile Lys Ile Glu Cys Val Leu Arg Glu Asn Tyr Arg Cys 1 5 10 15 ggg gag tcc cct gtg tgg gag gag gca tca aag tgt ctg ctg ttt gta 96 Gly Glu Ser Pro Val Trp Glu Glu Ala Ser Lys Cys Leu Leu Phe Val 20 25 30 gac atc cct tca aag act gtc tgc cga tgg gat tcg atc agc aat cga 144 Asp Ile Pro Ser Lys Thr Val Cys Arg Trp Asp Ser Ile Ser Asn Arg 35 40 45 gtg cag cga gtt ggtgta gat gcc cca gtc agt tca gtg gca ctt cga 192 Val Gln ArgVal Gly Val Asp Ala Pro Val Ser Ser Val Ala Leu Arg 50 55 60 cag tca gga ggc tat gtt gccacc att gga acc aag ttc

tgt gct ttg 240 Gln Ser Gly Gly Tyr Val Ala Thr Ile Gly Thr Lys Phe Cys Ala Leu 65 70 75 80aac tgg gaagat caa tca gta ttt atc cta gcc atg gtg gat gaa gat 288 Asn Trp Glu Asp Gln Ser Val Phe Ile Leu Ala Met Val Asp Glu Asp 85 90 95 aag aaa aac aatcga ttc aat gat ggg aag gtg gat cct gct ggg aga 336 Lys Lys Asn Asn Arg Phe Asn Asp Gly Lys Val Asp Pro Ala Gly Arg 100 105 110 tac ttt gct ggt acc atg gct gag gaa acc gcc cca gct gtt ctg gag 384 Tyr Phe Ala Gly Thr Met Ala Glu Glu Thr Ala Pro Ala Val Leu Glu 115 120 125 cgg cac caa ggg tcc ttg tac tcc ctt ttt cct gat cac agt gtg aag 432 Arg His Gln Gly Ser Leu Tyr Ser Leu Phe Pro Asp His Ser Val Lys 130 135 140 aaa tac ttt aac caa gtg gat atc tcc aat ggt ttg gat tgg tcc ctg 480 Lys Tyr Phe Asn Gln Val Asp Ile Ser Asn Gly Leu Asp Trp Ser Leu145 150 155 160gac cat aaa-atc-ttc-tac-tac att-gac-agc-ctg-tcc tac act gtg gat 528 Asp His Lys Ile Phe Tyr Tyr Ile Asp-Ser-Leu-Ser-Tyr Thr Val Asp 165 170 175 gcc ttt gac tat gac ctg cca aca gga cag att tcc aac cgc-agg-act 576 Ala Phe Asp Tyr Asp Leu Pro Thr Gly Gln Ile Ser Asn Arg Arg Thr 180 185 190 gtt tac aag atg gaa aaa gat gaa caa atc cca gat gga atg tgc att 624 Val Tyr Lys Met Glu Lys Asp Glu Gln Ile Pro Asp Gly Met Cys Ile 195 200 205 gat gtt gag ggg aag ctt tgg gtg gcc tgt tac aat gga gga aga gta 672 Asp Val Glu Gly Lys Leu Trp Val Ala Cys Tyr Asn Gly Gly Arg Val 210 215 220 att cgc cta gat cct gag aca ggg aaa aga ctg caa act gtg aag ttg 720 Ile Arg Leu Asp Pro Glu Thr Gly Lys Arg Leu Gln Thr Val Lys Leu225 230 235 240cct gtt gat aaa aca act tcatgctgc ttt gga ggg aag gat tac tct 768 Pro Val Asp Lys Thr Thr Ser Cys Cys Phe Gly Gly Lys Asp Tyr Ser 245 250 255gaa atg tac gtg aca tgt gcc agg gat ggg atg agc gcc gaa ggt ctt 816 Glu Met Tyr Val Thr Cys Ala Arg Asp Gly Met Ser Ala Glu Gly Leu 260 265 270 ttg agg cag cct gatgct ggt aac att ttc aag ata aca ggt ctt ggg 864 Leu Arg Gln Pro Asp Ala Gly Asn Ile Phe Lys Ile Thr Gly Leu Gly 275 280 285 gtc aaa gga att gct ccatat tcc tat gca ggg taa 900 Val Lys Gly Ile Ala Pro Tyr Ser Tyr Ala Gly 290 295 300 <210> 2<211> 299 <212> PRT <213> Rattus norvegicus <400> 2 Met Ser Ser IleLys Ile Glu Cys Val Leu Arg Glu Asn Tyr Arg Cys 1 510 15 Gly Glu Ser Pro Val Trp Glu Glu Ala Ser Lys Cys LeuLeuPhe Val 20 25 30 Asp Ile Pro Ser Lys Thr Val Cys Arg Trp Asp Ser Ile Ser Asn Arg 35 40 45 Val Gln Arg Val Gly Val Asp Ala Pro Val Ser-Ser-Val-Ala-Leu Arg 50 55 60 Gln Ser Gly Gly Tyr-Val-Ala-Thr-Ile Gly Thr Lys Phe Cys Ala Leu 65 70 75 80 Asn Trp Glu Asp Gln Ser Val Phe Ile Leu Ala Met Val Asp Glu Asp 85 9095 Lys Lys Asn AsnArg Phe Asn Asp Gly Lys Val Asp Pro Ala Gly Arg 100 105 110 Tyr Phe Ala Gly Thr Met Ala Glu Glu Thr Ala Pro Ala Val Leu Glu 115 120 125 Arg His Gln Gly Ser Leu Tyr Ser Leu Phe Pro Asp His Ser Val Lys 130 135 140 Lys Tyr Phe Asn Gln Val AspIle Ser Asn Gly Leu Asp Trp Ser Leu 145 150 155 160 Asp His Lys Ile Phe Tyr Tyr Ile Asp Ser Leu Ser Tyr Thr Val Asp 165 170 175 Ala Phe Asp Tyr AspLeuPro Thr Gly Gln Ile Ser Asn Arg Arg Thr 180 185 190 Val Tyr Lys Met Glu Lys AspGlu Gln Ile Pro Asp Gly Met

Cys Ile 195 200 205 Asp Val Glu Gly Lys Leu Trp Val Ala Cys Tyr Asn Gly Gly Arg Val 210 215 220 Ile Arg Leu Asp Pro Glu Thr Gly Lys Arg Leu Gln Thr Val Lys Leu 225 230 235 240 Pro Val Asp Lys Thr Thr Ser Cys Cys Phe Gly Gly Lys Asp Tyr Ser 245 250 255 Glu Met Tyr Val Thr Cys Ala ArgAsp Gly Met Ser Ala Glu Gly Leu 260 265 270 Leu Arg Gln Pro Asp Ala Gly AsnIlePhe Lys Ile Thr Gly Leu Gly 275 280 285 Val Lys Gly Ile Ala Pro Tyr Ser Tyr Ala Gly 290 295 <210> 3 <211> 24<212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence:Primer huRC -1<400> 3 ggaggctatg ttgccaccattgga 24 <210> 4 <211> 23<212> DNA <213> Artificial Sequence<220> <223>Description of ArtificialSequence:Primer huRC -2<400> 4 ccctccaaag cagcatgaag ttg 23

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing in the expression vector construction for transgenic rat production of this invention showing the process which starts an ORF part from the rat REGYUKA rutin overall length cDNA.
[Drawing 2] It is drawing in the expression vector construction for transgenic rat production of this invention showing the process which introduces the ORF part of the rat REGYUKA rutin overall length cDNA into an expression vector pCXN2.
[Drawing 3] It is drawing showing the process of introductory gene fragment preparation in which the linear rise was carried out for transgenic rat production of this invention.
[Drawing 4] It is drawing showing the location of the primer in the check by PCR of the REGYUKA rutin gene in the transgenic rat of this invention.
[Drawing 5] It is drawing using the pQTC bone density measuring apparatus for animal study showing the result of a scan of the femur organization (metaphysis section and diaphysis) of the bone symptoms model rat of this invention.
[Drawing 6] It is drawing using the pQTC bone density measuring apparatus for animal study showing the result of a scan of the femur organization (metaphysis section and diaphysis) of SD system wild type normal rat of contrast.

[Translation done.]